Targeting Huntingtin Expression in Patients with Huntington's Disease

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Abstract

Background

Huntington's disease is an autosomal-dominant neurodegenerative disease caused by a trinucleotide repeat (CAG) expansion in *HTT*, resulting in a mutant huntingtin protein. IONIS-HTT$_{Rx}$ is an antisense oligonucleotide designed to inhibit *HTT* messenger RNA and thereby reduce levels of mutant huntingtin.

Methods

We conducted a randomized, double-blind, multiple-ascending-dose, phase 1/2a study in adult persons with early-manifest Huntington's disease. Participants received IONIS-HTT$_{Rx}$ or placebo (3:1) via bolus intrathecal administration every 4 weeks for 4 doses. Dose selection was guided by a preclinical model in mouse and monkey relating dose level to reduction in huntingtin. The primary endpoint was safety. The secondary endpoint was IONIS-HTT$_{Rx}$ pharmacokinetics in cerebrospinal fluid. Prespecified exploratory outcomes included the concentration of mutant huntingtin in cerebrospinal fluid.

Results

Of the 46 patients who enrolled in the study, 34 were randomized to receive IONIS-HTT$_{Rx}$ (10 to 120 mg) and 12 were randomized to receive placebo; each participant received all 4 doses and completed the study. Adverse events were reported in 98% of patients with all being grade 1 or 2; no serious adverse events were seen in drug-treated patients. There were no clinically-relevant adverse laboratory parameter

changes. Pre-dose (trough) cerebrospinal fluid concentrations of IONIS-HTT$_{Rx}$ exhibited dose-dependence up to doses of 60 mg. IONIS-HTT$_{Rx}$ treatment resulted in dose-dependent reduction in cerebrospinal fluid mutant huntingtin (mean changes from baseline to endpoint of +9.8%, -19.9%, -25.0%, -27.5%, -42.4% and -37.7% for placebo, 10-, 30-, 60-, 90- and 120-mg dose groups, respectively).

Conclusions

Intrathecal administration of IONIS-HTT$_{Rx}$ to early HD patients was not accompanied by serious adverse events. We observed dose-dependent reductions in levels of mutant huntingtin.

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Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder inherited as an autosomal dominant trait, with onset typically in mid-adult life and characterized by movement disorder, cognitive decline and behavioral symptoms.[1] HD is caused by a CAG repeat expansion in the huntingtin (*HTT)* gene, which encodes huntingtin protein (HTT).[2] The abnormal gene results in production of gene products including mutant huntingtin protein (mHTT) containing an expanded polyglutamine tract, which causes neuronal dysfunction and death, putatively through toxic gain-of-function mechanisms.[3,4] Current treatments for HD are limited to symptomatic therapies, as no treatment has been shown to prevent onset or to slow progression. Given the monogenic nature of HD, we sought to inhibit *HTT* expression and thus directly target the primary disease mechanism.[5]

IONIS-HTT_{Rx} (also known as ISIS 443139 and RG6042; hereafter referred to as HTT_{Rx}) is a second-generation 2′-O-(2-methoxyethyl) (2′-O-MOE) antisense oligonucleotide designed to reduce levels of *HTT* messenger RNA (mRNA). HTT_{Rx} binds to its cognate mRNA through Watson-Crick base-pair interactions, triggering ribonuclease H1-mediated degradation of the target mRNA.[6] Antisense oligonucleotide-mediated selective reduction of *HTT* mRNA leads to lowered HTT levels and sustained amelioration of disease-associated phenotypes in multiple transgenic animal models of HD.[7] Chronic administration of *HTT*-lowering agents to wild-type non-human primates results in HTT reduction in central nervous system (CNS) tissues without adverse effects.[7,8] Experiments with alternative modalities designed to inhibit *HTT* expression yield similar effects in animal models of HD[8-10], validating HTT reduction as a potentially

viable disease-modifying therapeutic strategy. We report the results of a targeted HTT-lowering agent, in this Phase 1/2a clinical study of bolus intrathecally-administered *HTT*-targeting antisense oligonucleotide in adults with early HD.

Methods

Study drug

HTT_{Rx} is a chemically modified synthetic oligomer that is perfectly complementary to a 20-nucleotide stretch of *HTT* mRNA. HTT_{Rx} binds to *HTT* mRNA through Watson-Crick base pairing, with hybridization resulting in endogenous ribonuclease H1-mediated degradation of the *HTT* mRNA, inhibiting translation of the huntingtin protein. The sequence of HTT_{Rx} is (5' to 3') $cc_ot_oc_oa_ogTAACATTGACa_oc_oc_oac$, where capital letters represent 2'-deoxyribose nucleosides, and small letters 2'-(2-methoxyethyl)ribose nucleosides. Nucleoside linkages represented with a subscript $_o$ are phosphodiester, and all others are phosphorothioate. Letters represent adenine, 5-methylcytosine, guanine, and thymine nucleobases.

Study oversight

The study was conducted in accordance with the Declaration of Helsinki. The study protocol and all documentation were approved by the institutional review board or independent ethics committee at each investigational site. All patients provided written, informed consent. The study was sponsored by Ionis Pharmaceuticals, which provided the study medication (HTT_{Rx} and placebo). Personnel from Ionis designed the study in conjunction with collaborators from F. Hoffmann-La Roche Ltd, principal academic investigators and other disease experts. An independent data safety monitoring board authorized each dose escalation after unblinded review of safety data and consultation with the sponsor. The investigators collected the data, which was held and maintained by Ionis. Data were analyzed by personnel from Ionis and were interpreted by all

authors. The investigators vouch for the fidelity of the study to the protocol and protocol amendments. The authors vouch for the completeness and accuracy of the data. The authors and sponsor made the decision to submit the manuscript for publication.

Patients

Eligible participants were between the ages of 25 and 65 and had early manifest disease, defined by 36 or more CAG repeats in the *HTT* gene and clinical stage 1 disease (Unified Huntington's Disease Rating Scale total functional capacity scores of 11-13, where scores can range from 0 to 13 and 11-13 represents little to no functional impairment across the items assessed – occupation, finances, domestic chores, activities of daily living and care level).[11] Further details of the inclusion and exclusion criteria are provided in the Supplementary Appendix.

Study design and objectives

HTT_{Rx}-CS1 was a randomized, double-blinded, placebo-controlled, multi-center, Phase 1/2a, first-in-human study, performed at 9 centers in UK, Germany and Canada from August 2015 to November 2017. A centralized automated randomization system was used to assign patients in the ratio 3:1 to HTT_{Rx} or placebo within each of 5 dosing cohorts in an ascending-dose design (10 mg, 30 mg, 60 mg, 90 mg or 120 mg). Each participant received 4 bolus intrathecal injections of HTT_{Rx} or placebo (artificial cerebrospinal fluid) at 4-week intervals followed by a 4-month untreated follow-up period. A cerebrospinal fluid (CSF) sample was collected prior to each study drug administration and either 4 or 8 weeks after the last dose of study drug (Figure 1.)

Investigators, participants, and sponsor were unaware of the treatment assignments for the duration of the study.

The primary objective was evaluation of the safety and tolerability of HTT_{Rx}. Safety evaluations included physical examination, neurologic examination, Columbia-Suicide Severity Rating Scale, laboratory assessments, vital signs, electrocardiogram and safety neuroimaging sequences. At each study visit, participants were queried for other changes in health status in an open ended fashion.

The secondary objective was characterization of CSF pharmacokinetics of HTT_{Rx}. Exploratory objectives were characterization of plasma pharmacokinetics of HTT_{Rx} and exploration of the effects of HTT_{Rx} on pharmacodynamic biomarkers and clinical endpoints relevant in HD, including the concentrations of mutant huntingtin protein (mHTT) and neurofilament light protein in the CSF, ventricular volume and HD Cognitive Battery composite cognitive score. After the completion of the study, participants were offered the opportunity to enroll in a 15-month, open-label extension study (NCT03342053) evaluating the effects of either monthly or every other month intrathecal administration of 120 mg HTT_{Rx}.

Measurement of Cerebral Volume

3-Tesla T1-weighted structural brain MR scans were obtained and transferred to an independent image analysis provider where quality-control, processing and volumetric analysis were performed, blinded to treatment status, according to established methods.[12] Whole brain and regional volume changes were calculated using the

boundary shift integral, a semi-automated method that determines volume change from three-dimensional shift between paired images of a region's boundary.

Statistical analysis

The primary objective of the study was evaluation of safety. Treatment-emergent adverse events (AE), serious AEs, laboratory tests (blood and CSF), vital signs, ECG measures and observations from the Columbia Suicide Severity Rating Scale (C-SSRS) were summarized by treatment group. Where possible, pharmacokinetic parameters were assessed for HTT_{Rx} in CSF (secondary objective) and plasma (exploratory objective). Analyses of pharmacodynamic biomarkers and clinical endpoints were summarized by treatment group, and the HTT_{Rx}-treated groups were compared to placebo. The treatment differences and 95% confidence intervals (CIs) for changes in CSF mHTT were Hodges-Lehmann estimations based on Wilcoxon Rank Sum Test or obtained using ANOVA, depending on the normality of the data. Relationships between CSF mHTT reduction and clinical outcomes were explored in a post-hoc setting using the Spearman correlation coefficient, and the 95% CI of the correlation coefficient was based on Fisher's z transformation. Due to the exploratory nature of this study, adjustments for multiplicity of testing were not used. Interpretation of HTT_{Rx} effects on tissue mHTT is based on the extent of reduction of mHTT reduction in the CSF and a linked pharmacokinetic/pharmacodynamic clearance model based on data collected in human mHTT transgenic mice and non-human primate (details provided in the Supplementary Appendix).

Results

Patients

From August 2015 through April 2017, 52 patients were screened for eligibility and 46 patients underwent randomization according to the protocol. All patients received all scheduled doses of assigned treatment, and all randomized patients completed the study according to the protocol. (Patient flow diagram is provided as Supplementary Appendix Figure S3.) The baseline characteristics were representative of early-stage Huntington's disease and were similar across the treatment groups (Table 1).

Primary Objective -- Safety and Tolerability

The incidence of adverse events (AEs) was similar in patients receiving HTT_{Rx} and patients receiving placebo (Table 2). All events were mild or moderate in severity. The most commonly reported AEs in HTT_{Rx}-treated patients were procedural pain and post-dural puncture headache, which occurred after approximately 10% of lumbar punctures and had no apparent relationship to study duration or dose. There was no evidence for increased risk of post-dural puncture headache with successive lumbar punctures. All post-dural puncture headaches resolved (median duration of 2 days), and no blood patches were required. Very few AEs (6%) were considered related to study drug, and most related events (83%) were also considered related to study procedure. There were no deaths, dose-limiting AEs, treatment discontinuations or treatment delays during the study. The only serious AE was an inpatient admission of a patient in the placebo group with for observation of a mild post-dural puncture headache. Neither suicidal behavior nor serious suicidal ideation emerged in any patient during the study.

One case of mildly increased CSF leukocyte count (20-23 cells/mm^3, measured in triplicate) without associated symptoms was observed 8 weeks after last dose of 60 mg HTT_{Rx}; clinical safety MRI and EEG were normal. The asymptomatic elevation persisted throughout the post-treatment period and resolved prior to the patient's initiation of treatment in the extension study, 64 weeks after last dose in this study.

Secondary Objective

HTT_{Rx} was measurable in the CSF of most patients receiving doses of 30 mg or more. Trough concentrations increased with increasing dose from below the limit of quantification at the 10 mg dose through the 60 mg dose with a plateau in CSF concentration beyond 60 mg (Figure 2A). No accumulation was observed in CSF over time.

Exploratory Objectives

Plasma Concentrations of HTT_{Rx}

Median peak plasma concentrations were reached within 4 hours after bolus intrathecal administration and declined to less than 30% of peak concentration by 24 hours after dosing. HTT_{Rx} concentration in plasma increased approximately dose-proportionally over the explored dose range (Figure 2B), without evidence of plasma concentration accumulation 24 hours post-dose over the course of the study and a minor increase (<20%) in peak concentration at the 120 mg dose level.

Concentrations of mHTT in the Cerebrospinal Fluid

After four doses of HTT_{Rx} at 1 month intervals there were dose-dependent decreases in CSF mHTT concentration versus placebo, with maximal individual reduction of 63% and approximately 40% mean reduction for the 90- and 120-mg dose cohorts (Figure 3A, B; Table S1). Steady state maximal reduction of CSF mHTT does not appear to have been reached during the 3-month dosing period (Figure 3A,C).

Clinical Outcomes

Functional, cognitive, psychiatric and neurologic clinical outcomes were generally unchanged at the dose-group level during the study, and no differences were observed between placebo-treated patients and patients who received HTT_{Rx}, regardless of dose. (See Supplementary Appendix, Table S2.)

Ventricular volume exhibited dose- and time-dependent increases at Day 113 and at Day 197, without adverse consequences, in the 90-mg and 120-mg dose groups compared to the placebo group (boundary shift intervals of 2.6 and 5.0 mL for the 90-mg group and 2.3 and 5.3 mL for the 120-mg group at Days 113 and 197, respectively). Elevations of the neurofilament light protein in the CSF occurred in some high-dose patients at Day 113 or 141 (i.e., one or two months after cessation of dosing, respectively) without associated adverse events or safety MRI changes. (See Supplementary Appendix Figure S4.) By the start of the extension study (7-27 months since final doses in this study), levels of the neurofilament light protein in the CSF had generally returned to pre-study levels; during the extension study they rose with a similar time course and magnitude as observed in this study and then decreased at later timepoints despite continued treatment (unpublished data).

Post-hoc Analyses

In parallel with this study, the composite Unified Huntington Disease Rating Scale (cUHDRS) was developed to serve as a measure of clinical progression in stage I and II HD.[13] We examined the relationships between the degree of CSF mHTT lowering and changes in the cUHDRS and its four components and observed correlations between reduction in CSF mHTT and improvements in cUHDRS and two of its components (Supplementary Appendix Figure S5). These correlations should be interpreted with caution, because the tests were not prespecified, nor the coefficients of correlation adjusted for multiple testing.

Discussion

Four repeated monthly bolus intrathecal administration of HTT_{Rx}, an *HTT* mRNA-targeting antisense oligonucleotide, to adults with early HD was not accompanied by any serious adverse events. The intervention achieved a dose-dependent reduction of mHTT, the protein that putatively causes HD, in the CSF. Based only on this study, we do not know whether this reduction reflects a reduction of mHTT in the CNS, although preclinical studies support the hypothesis that CSF mHTT levels reflect CNS tissue mHTT level (Supplemental Appendix and Southwell et al.[14]). While the positive effects of sustained lowering of mHTT on motor function and survival in mouse models of HD[7,8] provided a rationale for development of an HTT-targeting antisense oligonucleotide, larger studies of greater duration are needed to determine whether HTT_{Rx}-mediated reduction of mHTT in the CSF is associated with a treatment effect on disease course,

which is typically slow, with changes on standard outcomes generally occurring over years.

Ventricular volume exhibited apparent dose- and time-dependent increases during the study without corresponding changes in whole brain volume. Slow, progressive whole brain atrophy (i.e., irreversible loss of brain tissue) and ventricular expansion are characteristic features of HD,[15] and neuroinflammation is a known phenomenon in HD.[16,17] Although "pseudoatrophy" (i.e., ventricular expansion due to resolution of inflammatory edema and gliosis) has been described in clinical studies of multiple sclerosis and Alzheimer's disease, it has been a challenge to differentiate between treatment-induced pseudoatrophy and disease-related atrophy,[18-23] and we have not assessed the effect of HTT_{Rx} treatment on inflammation or gliosis has been conducted in humans or animal models.

The putative neuronal injury marker, the neurofilament light protein in the CSF,[24] exhibited apparent dose- and time-dependent increases during the study, and reversed after cessation of study treatment and also after transient increases during the extension study. To our knowledge, there are no published longitudinal studies of neurofilament light protein in the CSF of persons with HD and so the magnitude of increase that corresponds with an adverse outcome is unknown. The values observed in this study are within the range observed in a cross-sectional study of HD patients.[25]

In sum, we have shown that the antisense oligonucleotide drug HTT_{Rx} reduces mHTT in the CSF of persons with HD. More generally, we have demonstrated

antisense-mediated protein suppression in the central nervous system of patients with a neurodegenerative disease.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Figure Legends

Figure 1. Study design. At the conclusion of the screening period, eligible patients were randomized 3:1 to HTT_{Rx} or placebo. CSF was collected prior to study drug administration on Days 1, 29, 57 and 85; the CSF sample on Day 1 serves as a baseline sample, and CSF samples on Days 29, 57 and 85 serve as 28-day post-dose trough samples. In each patient, one sample was collected after completion of dosing, either on Day 113 or Day 141 according to randomized assignment. CSF collection on Day 113 serves as a 28-day post-last-dose sample; CSF collection on Day 141 serves as a 56-day post-last-dose sample. Dotted lines indicate the relationship between each dose and the subsequent CSF sample.

Figure 2. HTT_{Rx} exposure (A) Maximum pre-dose (i.e., 28-day trough) HTT_{Rx} concentration in CSF by dose group. (B) Mean ± SEM HTT_{Rx} concentration in plasma by dose group over the 24-hour periods after the first dose (left) and fourth dose (right). See Supplemental Appendix for further discussion of observed HTT_{Rx} concentrations.

Figure 3. Effect of HTT_{Rx} on CSF mHTT concentrations (A) CSF mHTT levels over time (absolute values in fM, top; percent change from baseline, bottom) for individual patients in each dose group. Arrowheads indicate dosing days. See Supplemental Appendix for a discussion of individual patient data observed in the 120mg dose group. (B) CSF mHTT percent change from baseline to last available 28-day post-dose timepoint (i.e., either Study Day 113 for patients who underwent CSF sampling at Study Day 113 or Study Day 85 for patients who did not) for individual patients (circles) and

dose group means (horizontal lines). (C) Mean ± SD absolute change (left) and percentage change from baseline over time by dose group. Arrowheads indicate dosing days. As shown by dotted lines and as illustrated in Figure 1, Day 113 and Day 141 samples were each performed in a randomized subset of patients.

Tables

Table 1. Patient characteristics at baseline

	Placebo (n=12)	**HTT_{Rx}, all (n=34)**	**HTT_{Rx}, 10mg (n=3)**	**HTT_{Rx}, 30mg (n=6)**	**HTT_{Rx}, 60mg (n=6)**	**HTT_{Rx}, 90mg (n=9)**	**HTT_{Rx}, 120mg (n=10)**
Age, yr	49±10	46±10	44±17	53±7	43±11	46±10	45±10
Female sex, no. (%)	4 (33)	14 (41)	1 (33)	1 (17)	3 (50)	3 (33)	6 (60)
White race, no. (%)	11 (92)	32 (94)	3 (100)	5 (83)	6 (100)	9 (100)	9 (90)
CAG repeat length	44±2	44±3	46±6	43±2	45±2	44±3	45±4
MoCA score	25±2	26±3	26±4	27±2	26±3	26±3	26±3
TFC score, no. (%) 11 12 13	6 (50) 4 (33) 2 (17)	9 (26) 15 (44) 10 (29)	0 (0) 1 (33) 2 (67)	2 (33) 4 (67) 0 (0)	2 (33) 3 (50) 1 (17)	2 (22) 4 (44) 3 (33)	3 (30) 3 (30) 4 (40)
TMS	24±7	22±10	21±7	20±13	25±13	22±10	21±9
Independence Scale	89±8	90±8	93±6	88±11	86±8	93±8	90±6
Disease burden score	398.4±50.1	383.7±66.0	385.2±109.1	366.7±50.8	383.8±34.3	364.5±68.7	410.8±75.1

mHTT in CSF, fM	109±43	110±46	144±50	120±45	117±30	105±65	96±35

Values with ± are mean ± SD; TFC, Unified Huntington's Disease Rating Scale Total Functional Capacity; MoCA, Montreal cognitive assessment; TMS, total motor score; disease burden score (calculated by [CAG repeat length - 35.5] * age in years) [26]; mHTT, mutant huntingtin protein concentration.

Table 2. Treatment-emergent adverse events reported in 3 or more patients in the HTT_{Rx} groups*

	Grade 1		Grade 2		Grade 3/4	
	HTT_{Rx} Groups (N = 34) n (%)	**Placebo Group (N = 12) n (%)**	**HTT_{Rx} Groups (N = 34) n (%)**	**Placebo Group (N = 12) n (%)**	**HTT_{Rx} Groups (N = 34) n (%)**	**Placebo Group (N = 12) n (%)**
Any adverse event	20 (58.8)	7 (58.3)	13 (38.2)	5 (41.7)	0	0
Any serious adverse event	0	1 (8.3)	0	0	0	0
System Organ Class Preferred Term						
Injury, poisoning and procedural complications	19 (55.9)	7 (58.3)	7 (20.6)	4 (33.3)	0	0
Procedural pain	17 (50.0)	4 (33.3)	2 (5.9)	2 (16.7)	0	0
Post lumbar puncture syndrome	8 (23.5)	4 (33.3)	4 (11.8)	1 (8.3)	0	0
Fall	7 (20.6)	2 (16.7)	0	1 (8.3)	0	0
Skin abrasion	5 (14.7)	1 (8.3)	0	0	0	0
Infections and infestations	21 (61.8)	4 (33.3)	2 (5.9)	2 (16.7)	0	0
Nasopharyngitis	7 (20.6)	0	0	2 (16.7)	0	0
Upper respiratory tract infection	3 (8.8)	1 (8.3)	1 (2.9)	0	0	0
Bronchitis	2 (5.9)	0	1 (2.9)	0	0	0
Influenza	2 (5.9)	0	1 (2.9)	0	0	0
Rhinovirus infection	3 (8.8)	0	0	0	0	0
Nervous system disorders	9 (26.5)	4 (33.3)	3 (8.8)	3 (25.0)	0	0
Headache	4 (11.8)	3 (25.0)	2 (5.9)	3 (25.0)	0	0
Hypoesthesia	3 (8.8)	0	0	0	0	0
Musculoskeletal and connective tissue disorders	9 (26.5)	4 (33.3)	1 (2.9)	1 (8.3)	0	0
Arthralgia	4 (11.8)	2 (16.7)	0	0	0	0
Back pain	3 (8.8)	1 (8.3)	1 (2.9)	0	0	0
General disorders and administration site conditions	5 (14.7)	2 (16.7)	1 (2.9)	0	0	0
Fatigue	4 (11.8)	0	1 (2.9)	0	0	0
Gastrointestinal disorders	5 (14.7)	1 (8.3)	1 (2.9)	0	0	0
Toothache	2 (5.9)	0	1 (2.9)	0	0	0
Vascular disorders	3 (8.8)	0	0	0	0	0
Hematoma	3 (8.8)	0	0	0	0	0

* Each adverse event was rated as mild, moderate or severe, corresponding to grade 1, 2 or 3, respectively. In addition, serious adverse events were rated as life-threatening (grade 4) or not life-threatening. At each level of summation (overall, System Organ Class, Preferred Term), patients reporting more than one adverse event are counted only once using the most severe category.